LIBRARY OF PH RESPONSIVE POLYMERS AND NANOPROBES THEREOF

[0001] This application is a continuation of U.S. application Ser. No. 16/114,187, filed Aug. 27, 2018, which is a continuation of U.S. application Ser. No. 15/369,701, filed Dec. 5, 2016, now U.S. Pat. No. 10,098,971, which is a continuation of International Application No. PCT/US2015/034575, filed Jun. 5, 2015, which claims the benefit of priority from U.S. Provisional Application Ser. No. 62/009,019, filed on Jun. 6, 2014, the entire contents of each of which are incorporated herein by reference.

[0002] This invention was made with government support under Grant Number R01 EB013149 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

1. Field

[0003] The present disclosure relates generally to the fields of molecular and cellular biology, cancer imaging, nanotechnology, and fluorescence sensors. More particularly, it relates to nanoplatforms for the detection of pH changes.

2. Description of Related Art

[0004] Fluorescence imaging has become an important tool in the study of biological molecules, pathways and processes in living cells thanks to its ability to provide spatial-temporal information at microscopic, mesoscopic and macroscopic levels (see, e.g., Tsien, R. Y. *Nat. Rev. Mol. Cell Biol.* 2003, 4, SS16; Weissleder, R., *Nature* 2008, 452, 580; Fernandez-Suarez, *M., Nat. Rev. Mol. Cell Biol.* 2008, 9, 929). Recently, activatable imaging probes that are responsive to physiological stimuli such as ionic and redox potentials, enzymatic expressions, and pH have received considerable attention to probe cell physiological processes (see, e.g., de Silva, A. P., Chem. Rev. 1997, 97, 1515; Zhang, J., *Nat. Rev. Mol. Cell Biol.* 2002, 3, 906; Fee, S., *Chem. Commun.* 2008, 4250; Kobayashi, H.; *Chem. Res.* 2010, 44, 83; Fovell, J. F., *Chem. Rev.* 2010, 110, 2839; Ueno, T., *Nat. Methods* 2011, 8, 642). Among these stimuli, pH stands out as an important physiological parameter that plays a critical role in both the intracellular (pH_i) and extracellular (pH_e) milieu (Alberts, B., *Molecular Biology of the Cell;* 5th ed.; Garland Science: New York, 2008).

[0005] Although various pH-sensitive fluorescent probes have been reported (Kobayashi, H., *Chem. Rev.* 2010, 110, 2620; Han, J. Y., *Chem. Rev.* 2010, 110, 2709), their pH sensitivity primarily arises from ionizable residues with pH-dependent photo-induced electron transfer (PeT) properties to the fluorophores. One potential drawback for these fluorescent agents is their broad pH response (ΔpH~2) as dictated by the Henderson-Hasselbalch equation (Atkins, P., Physical Chemistry, Oxford University Press, 2009). This lack of sharp pH response makes it difficult to detect subtle pH differences between the acidic intracellular organelles (e.g., <1 pH difference between early endosomes and lysosomes) (Maxfield, F. R., *Nat. Rev. Mol. Cell Biol.* 2004, 5, 121; Casey, J. R., *Nat. Rev Mol. Cell Biol.* 2010, 11, 50) or pHe in solid tumors (6.5-6.9) (Webb, B. A., *Nat. Rev. Cancer* 2011, 11, 671; Zhang, X., *J. Nucl. Med.* 2010, 51, 1167.) over normal tissue environment (7.4). Moreover, simultaneous control of pH transition point and emission wavelengths (in particular, in the near IR range) is difficult for small molecular dyes. Recent attempts to develop pH-sensitive fluorescent nanoparticles primarily employ polymers conjugated with small molecular pH-sensitive dyes (Srikun, D., *J. Chem. Sci.* 2011, 2, 1156; Benjaminsen, R. V., *ACS Nano* 2011, 5, 5864; Albertazzi, L., *J. Am. Chem. Soc.* 2010, 132, 18158; Urano, Y., *Nat. Med.* 2009, 15, 104) or the use of pH-sensitive linkers to conjugate pH-insensitive dyes (Li, C., *Adv. Eunct. Mater.* 2010, 20, 2222; Almutairi, A., *J. Am. Chem. Soc.* 2007, 130, 444.). These nanoprobe designs also yield broad pH response and lack the ability to fine-tune pH transition point.

[0006] Recently, the use of polymers to create a pH responsive system has been described in WO 2013/152059, which produces a relatively narrow range of pH transition points based upon the specific monomer used but lacks the flexibility to fine-tune the pH transition point specifically.

[0007] Furthermore, imaging of tumor cells can provide enhanced methods of delineating the tumor boundaries and increasing the efficacy of surgery to resect a tumor. A variety of methods have been proposed to assist in the delineation of tumor boundaries. Conventional imaging modalities such as CT, MRI or ultrasound using image navigators such as the Brainlab™ first use pre-operative images followed by the intra-operative use of surgical fiducial markers to guide resection of skull base and sinus cancers as well as brain tumors. A major drawback is that only tumors that are immobile relative to firm bony landmarks can be accurately imaged and the pre-operative images cannot be updated to account for intra-operative manipulations to provide real-time feedback. Intra-operative MRI is being used in a few centers for imaging brain tumors but requires expensive installation of magnets into the operative suite for real time imaging and a recent review suggest that this may be of marginal benefit over conventional surgical navigation (Kubben et al., 2011). Ultrasound has been used to assess tumor depth for oral cavity HNSCC but is difficult to use in less accessible primary sites of the head and neck (Lodder et al., 2011).

[0008] These anatomy-based imaging modalities have great resolution but provide little disease specific information. Optical imaging strategies have rapidly been used to image tissues intra-operatively based on cellular imaging, native autofluorescence, and Raman scattering (Vahrmeijer et al., 2013; Nguyen & Tsien, 2013; Dacosta et al., 2006; Draga et al., 2010; Haka et al., 2006; Schwarz et al., 2009 and Mo et al., 2009). Unfortunately, using tissue autofluorescence for tumor margin detection is limited by high false positive and false negative results due to the lack of robust spectroscopic differences between cancer and normal tissues (Liu et al., 2010; Kanter et al., 2009; Ramanujam et al., 1996 and Schomacker et al., 1992).

[0009] A variety of exogenous fluorophores have been developed for intra-operative margin assessment. Most common strategies have focused on cell-surface receptors such as folate receptor-α (FR-α) (van Dam et al., 2011), chlorotoxin (Veiseh et al., 2007), epidermal growth factor receptor (EGFR) (Ke et al., 2003 and Urano et al., 2009), Her2/neu (Koyama et al., 2007), tumor associated antigens (e.g., prostate-specific membrane antigen, PSMA) (Tran Cao et al., 2012, carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) (Tran Cao et al., 2012; McElroy et al., 2008). Among these, folate-FITC and chlorotoxin-Cy5.5